METHODS AND EFFECTS OF FERTILITY MANAGEMENT: CHAPTER 4

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My Background

- Master's and PhD degrees studying domestic horse reproductive physiology and behavior with O.J. Ginther, University of Wisconsin-Madison
- BLM contract to University of Minnesota late 1980s to test vasectomy in band stallions and steroid hormone implants in mares in Nevada and southeastern Oregon (NRC report 1991)
- 25 years as director of the contraception program for the Association of Zoos and Aquariums (AZA)
- Co-editor of the book, *Wildlife Contraception: Issues, Methods, and Applications*
- Scientific advisor to Alliance for Contraception in Cats & Dogs

Fertility Control for Population Management

An increasingly popular approach to managing free-ranging populations

Active research and development underway for a wide variety of species and situations, e.g.,

- White-tailed deer
- Feral pigs
- Feral cats
- Stray dogs
- Coyotes

Horse Basics

At what points in the reproductive process fertility control can be applied

How the methods might affect natural processes, particularly behavior and the social system

Behavior and Social Structure

Preserving natural behavior was a criterion considered important in weighing various approaches to fertility control

- Basic social unit is the harem, with several mares plus one dominant stallion, sometimes including a subordinate stallion
- Breeding is seasonal, extending from spring through late summer or early fall

Stability of bands (harems) varies among locations, and some degree of turnover appears natural

- Stallions remain with their harems through the non-breeding season
- Offspring disperse at sexual maturity, with young males joining bachelor groups and young females joining other harems

Reproductive Physiology

- Puberty at about 18 months for both males and females
- But males don't usually gain access to females for mating for several more years
- Mares give birth in the spring, followed about a week later by post-partum estrus
- They continue to cycle approximately every 3 weeks during lactation until pregnant again, but interbirth intervals for free-ranging horses seems to be about 2 years, probably limited by nutritional status

Fertility rates and gestation

- Fertility rates can be high, with 80-100% conception rates in domestic horses in good condition
- Fertility tends to be lower in young and old mares
- With a gestation length of 11 months, mares in good condition can produce a foal every year

The Potential Fertility Control Methods

General Caveat:

There is no method for preventing production of foals that doesn’t affect some aspect of physiology or behavior

General Considerations

- Male- or female-directed
- Permanent or reversible
- Percentage efficacy
- Side effects (transient or chronic)
  - Safety
  - Effects on behavior
- Duration of action and reversibility rate
- Delivery route: remote delivery or requiring capture
- Availability of the product

THE METHODS

Adjustment of sex ratio

- Selective removal of females: outcome would probably be increased rates of fighting among males for limited number of females
- Selective sterilization of males: castration of "excess" males would likely result in an increase in the number of bachelor groups, but castrated males probably wouldn't be able to compete with intact stallions and be ostracized
- Selective reversible contraception of one gender: Outcome would depend on effect of the method on social interactions and competition

Female-Directed Methods

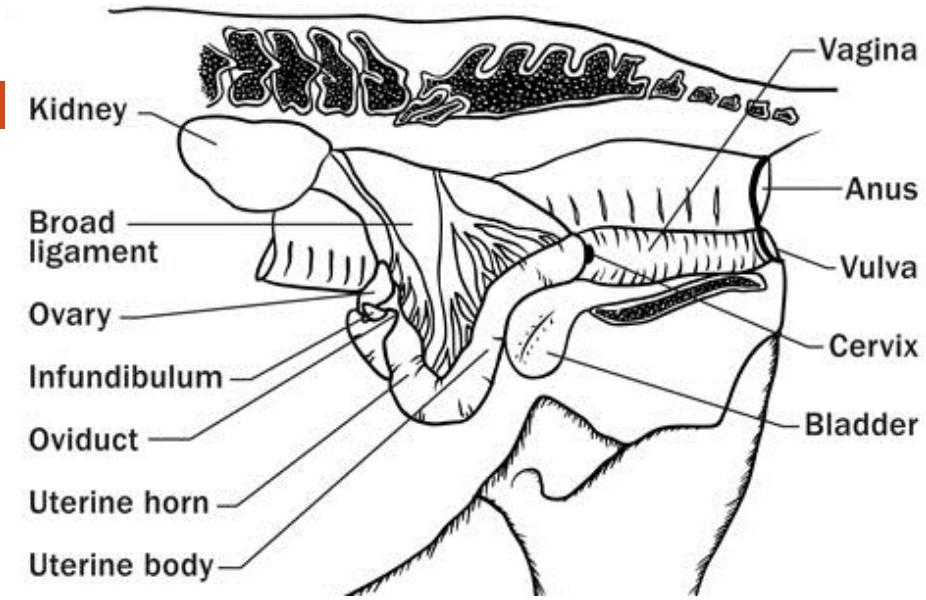
Permanent Sterilization

The committee was concerned about recommending surgical methods to be used in the field

- Surgical methods would require a vet
- Surgical methods are more likely to result in complications that could be serious or even fatal, such as bleeding or infection
- BLM staff could be trained to administer chemical sterilization methods

Permanent methods can affect population genetics

Permanent Methods for Females



Surgical Ovariectomy

Approaches:

- Abdominal
- Vaginal
- Laparoscopic

Involves major surgery that enters the body cavity

- Risk of complications, e.g., blood loss or infection post-surgery
- The Committee didn't feel this approach was advisable under field conditions, with its higher risk of contamination of the surgical site and limited opportunity for post-operative observation and care

Outcomes of Ovariectomy

Estrous/ovulatory cycles would be eliminated, so behavior would be altered

- Effects on harem integrity are unknown

In the non-breeding season or after ovariectomy, some domestic mares do exhibit irregular periods of estrous behavior

- This hasn't been reported in non-breeding season in free-roaming mares
- Studies of field ovariectomy haven't been reported

Other species

Trap-Neuter-Release programs for feral cats have not been successful at the population level

Very labor-intensive, so only reach a small percentage of the population

Especially true for castrated males:

Males no longer compete for territories and access to females, so any remaining intact males fill the void

Reversible Methods for Females

IMMUNOCONTRACEPTIVES

Porcine Zona Pellucida (PZP) Vaccines

The Different PZP Formulations

Earliest version: liquid PZP (now ZonaStat-H), recently registered with EPA for use in wild horse populations

- Highest efficacy when given as 2 injections, 1 month apart
- This treatment regimen is impractical in the field

PZP-22: more recent version, with time-release pellets to extend efficacy

- Prior testing showed good efficacy for about 2 years
- More recent test results showed significantly lower efficacy in second year

The third PZP formulation

SpayVac®

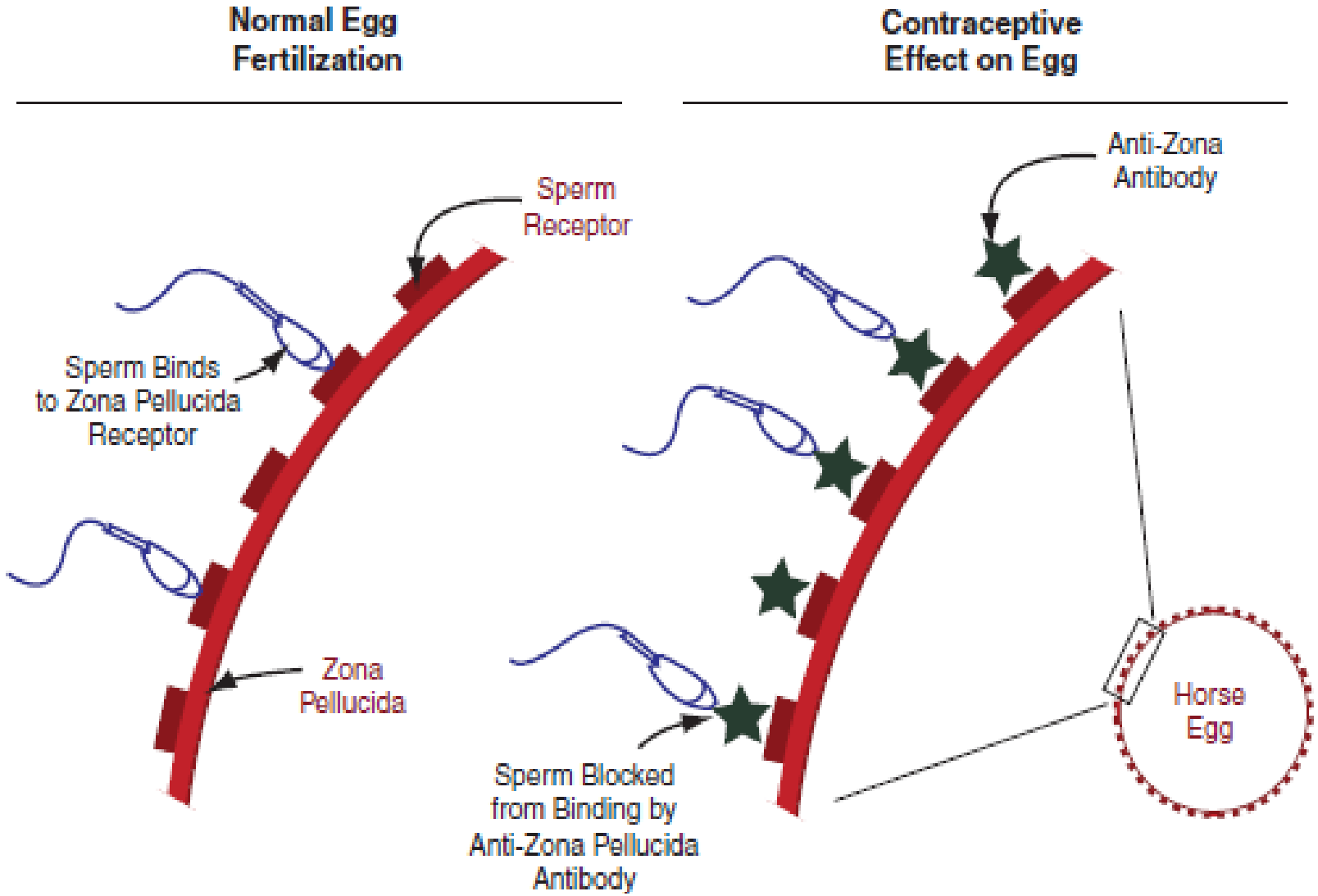
- Produced in Canada by ImmunoVaccine Technologies
- Requires only a single injection, but may be effective for up to 10 years

Only one published study with wild horses

- Results showed continued evidence of estrous behavior and other signs of estrogen production, indicating follicle growth
- But no evidence of ovulation, which would be needed for fertilization
- A study of safety using wild horses is underway (Univ. of Oregon) but results aren't yet available

PZP Mode of Action

PZP antibodies prevent sperm binding to the egg



Outcomes of PZP immunocontraception

Mares should continue to have estrous/ovulatory cycles without conception, so natural courtship and mating behavior continue

But because mares don't conceive, they undergo more estrous cycles than non-treated mares

Some concern that this might disrupt band integrity and cause mares to lose body condition

However, results from Assateague Island, where mares have been treated with liquid PZP for many years, show that:

- Treated mares are in better body condition
- And they live longer than ever reported for that population

Administering Vaccines

Liquid PZP and SpayVac can be delivered by dart

But efficacy has been higher when hand-injected

When darting

- Full dose may not be delivered
- Efficacy is higher when given in hip muscle

PZP-22 should be hand-injected to ensure sufficient pressure to inject the pellets

Darting may be practical in some HMAs, but gathering for hand-injection may be preferable in others

Efficacy of vaccines depends on

- Delivery of full, proper dose
- Adjuvant used
- Treatment protocol
- Individual differences of the animals
  - Genetic factors
  - Nutritional status
  - Immune status

The same factors will affect duration of effect, i.e., time to reversal

In addition, number of treatments affects reversal potential; may be well beyond the typical time in some mares

After repeated treatment with PZP, mares become less likely to recover and return to fertility

This might be considered an advantage or disadvantage

These and many “PZP” results are for the first two formulations

Less has been published about SpayVac

Time to reversal

When reversing, first pregnancy may result in giving birth late in season

- If conditions are harsh, this may jeopardize survival of foals through winter

- Data indicate that these mares return to early season births in subsequent years

Efficacy

Liquid PZP

Variable results for varying situations

But general efficacy rate about 90%

PZP-22:

Designed to release vaccine into the system at 1, 3, and 12 months post-injection

- Thus, best to treat in winter to increase the chances it will still be effective the second breeding season
- Some trials showed 85% efficacy in year 2,
- But more recent results were not as promising

Variability in PZP-22 results

According to Turner:

- Time of year of injection
- Hand-injection vs darting
- Location of injection (hip considered ideal)
- Possible differences in field preparation
- Change in production protocol
  - Heat extrusion
  - Cold evaporation

Efficacy (cont.)

SpayVac

Only one published trial in horses

- 100% efficacy year 1
- 83% years 2-4

Very promising, but not known if these results would hold in other situations and locations

Side effects of PZP vaccines

Pure formulations (made from oocytes separated from ovarian tissue) result in specific vaccines that should have no effects outside the ovary

Ovarian effects may result in less regular estrous cycles or disappearance of cycling

- This may become more likely with repeated treatments

Injection-site reactions

- Early liquid PZP used Freund's Complete Adjuvant, which caused injection-site reactions
- Now formulated with Freund's Modified Adjuvant, which is less likely to cause reactions
- Also, injecting in hip lowers likelihood of reactions

Other features

PZP vaccines can be given safely

- during pregnancy
- During lactation
- Before puberty

Genetic selection on immune response

Hypothesis: Mares that don't respond to the vaccines will be the ones reproducing and thus pass that resistance to their foals

- Over generations this would select for resistant animals, rendering PZP vaccine treatment ineffective
- Perhaps more seriously, this selective pressure might involve other immune functions, leaving the animals more susceptible to particular pathogens
- However, in the decades of treatment on Assateague Island, no such effects have been noted

Positive Effects

- Mares treated with liquid PZP vaccines on Assateague Island are in better body condition and live longer than untreated mares
- Presumably because they don't face the energetic demands of gestation and lactation

- This effect has more recently been reported for Shackleford Banks horses as well

Long-term population effects

Beyond target numbers of animals to treat and percentage efficacy

Treated mares live longer (increased population size)

Number of treatments is negatively associated with probability of future fertility

Modeling of these effects at the population level would be very helpful

Concern about SpayVac

The only published report of SpayVac treatment of wild horses

- Uterine edema

But this also occurs naturally during estrus, then regresses

Question is whether it would be chronic and result in pathology

A Different Type of Vaccine: Anti-GnRH

GnRH (Gonadotropin Releasing Hormone):

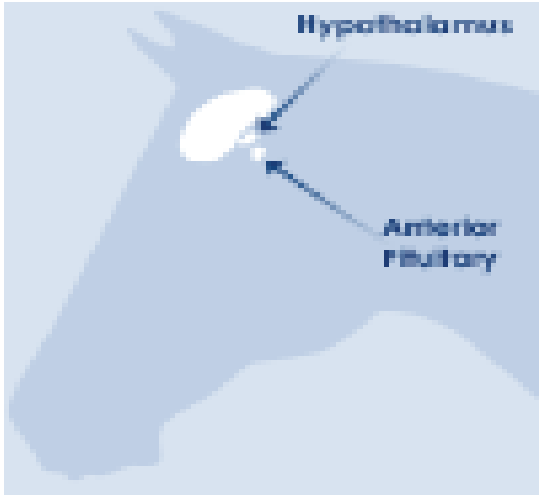
- Hypothalamic hormone that stimulates the pituitary to release LH and FSH that then stimulate the ovaries or testes
- Blocking GnRH or its action stops the reproductive process
- These hormones (GnRH, LH & FSH) are the same in both males and females
- So this can be effective in either gender

GonaCon®

Vaccine against GnRH

- Produced by NWRC in Ft. Collins, CO
- Registered by EPA for use in wild horses
- But not yet much published data for use in horses
- Can be delivered by dart

There are other GnRH vaccine products approved for horses in other countries; effects are likely similar



Normal

Hypothalamus

GnRH

Capillary Membrane

Pituitary Gland

LH

FSH

Ovary

Progesterone

Estrogen

Contraceptive Effect (GnRH vaccine injected)

Hypothalamus

GnRH

Capillary Membrane

Antibodies form and bind with GnRH.

GnRH-antibody complexes are too large to diffuse through membrane to pituitary gland

Pituitary Gland

LH

FSH

Ovary

Progesterone

Estrogen

GonaCon for horses

Two horse studies: one penned, one free-ranging

- Efficacy high in year 1, decreasing over next several years
- Efficacy was higher in pen study
- Used different methods of measuring efficacy
- Free-ranging mares were in poorer body condition, so may not have mounted as strong an immune response

Outcomes for GonaCon

- No effects on behavior found in field study
- Continuing signs of estrus in both studies
  - This suggests incomplete suppression of estrogen production despite effective contraception
  - Probably because estrogen is produced by growing follicles under stimulation from pituitary FSH

    FSH is stimulated by GnRH, but it also is secreted independent of GnRH
  - This can be an advantage to the extent that estrous behavior can be maintained

Use in Males and/or Females?

Males:

Because of the profound behavioral changes expected (equivalent to castration), the Committee didn't consider it as promising for use in wild stallions

FSH alone doesn't support either spermatogenesis or testosterone production

So testosterone likely to be completely suppressed

GnRH Agonists

An agonist is a synthetic form of a hormone that has a similar action on receptors in target tissues

GnRH vaccines block the action of GnRH

GnRH agonists stimulate pituitary release of LH & FSH

- This stimulatory effect can induce estrus and ovulation
- Continued treatment (as with an implant) causes down-regulation of pituitary receptors for GnRH, so no further LH or FSH is released

GnRH agonist

Suprelorin®: deslorelin acetate implants

- Fast-release implants (few days) are used routinely in mares to induce estrus and ovulation
- Return to cycling is delayed in some, suggesting that long-term treatment could be contraceptive
  - Effects could either be similar to GnRH vaccines
  - Or estrus could be completely suppressed
  - Slow-release implants re effective a minimum of 6 or 12 months, but considerable variability among individuals in duration of suppression (data from other species)

Suprelorin listed in U.S. only for ferrets

- Available only as a research product for other species
- No apparent advantage over GnRH vaccine such as GonaCon

Steroid Hormones

Estrogens and/or Progestins

Natural and synthetic progestins

Synthetic versions of natural hormones are produced to increase bioactivity (need smaller dose) and increase resistance to metabolism (deactivation) to extend action

There are MANY synthetic progestins, but only one, altrenogest, is effective in horses

Steroid hormones

- Can be incorporated into silastic implants for long-lasting effect
- But they can accumulate in body fat and thus can enter the food chain
- Thus, EPA (the regulatory body for field application of drugs) is not likely to approve use of steroids in wild horses

Mode of action

Estrogen and progesterone are part of the natural reproductive process

- However, with continual release at high concentrations, they can act as contraceptives and prevent ovulatory cycles via negative feedback (analogous to pregnancy)
  - Examples of human birth-control products:
    - Birth-control pill: estrogen plus progestin
    - Depo-Provera: progestin

Progesterone

Studies with natural progesterone in silastic implants in mares

- Implants in neck were lost
- Implants in peritoneal cavity were not successful, perhaps because dose was too low

Altrenogest hasn't been used as a contraceptive in mares, only to control and time estrous

Synthetic estrogen

Intra-peritoneal high-dose implants were effective

But the number of side effects of estrogen make it unsuitable:

- Chronic uterine edema
- Tissue growth stimulation leading to tumors and cancer
- Behavioral effects
  - Male-type behavior with long-term treatment
  - Interference with lactation

Synthetic estrogen + progesterone

Estrogen is usually paired with a progestin to counter its hyperstimulatory effects

The combination is also a more effect contraceptive than progestin alone

- In horse studies, intra-peritoneal implants were effective for 2-3 years

Intra-Uterine Devices (IUDs)

Two studies with horses

- IUDs commercially made for humans fell out
- Custom-made IUDs (correct size) were effective
  - Uterine inflammation during treatment reversed following removal

- However, there is no known source for horse IUDs

Male-Directed Methods

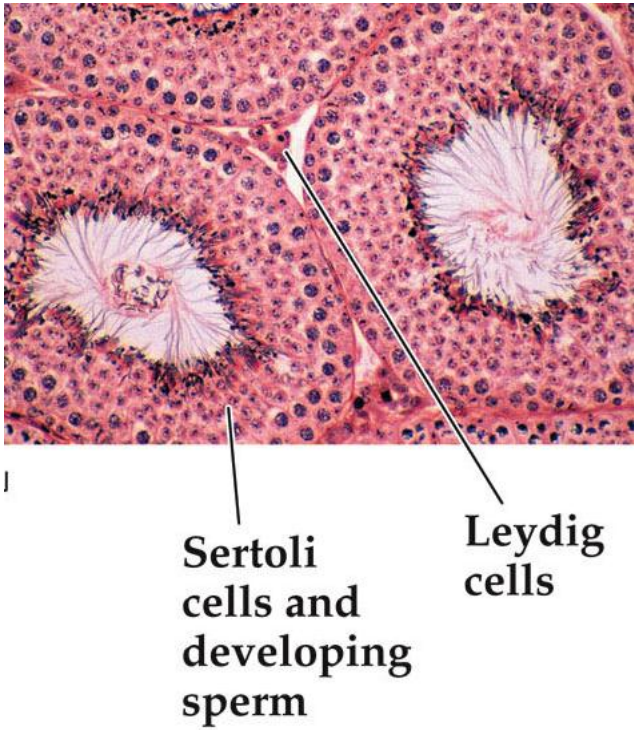
Permanent Methods for Males

Surgical or Chemical Castration

Surgical procedure is well established: gelding

Product for chemical sterilization is newer: Esterilsol®

- Zinc gluconate with arginine injected into the testis
- Destroys seminiferous tubules that make sperm
- May not completely eliminate testosterone production
- Primary application is stray domestic dogs



Outcomes of Surgical Castration

- Very effective in eliminating fertility for the males that are treated
- Likely to reduce or eliminate male-typical behavior
  - Individual differences in response to castration, but in general castrated males cannot compete well with intact males, so not likely to be able to acquire or keep a harem
- Foal production may not be affected, since remaining intact males will likely monopolize and inseminate females

Outcomes of Chemical Castration

- Effectiveness at eliminating fertility in stallions is not established; effectiveness is high in dogs
- Not possible to predict effect on male-type behavior, since testosterone may not be suppressed in all individuals
- If enough testosterone is produced, some treated stallions might be able to compete successfully with intact stallions and monopolize mares, which would prevent conception in those mares

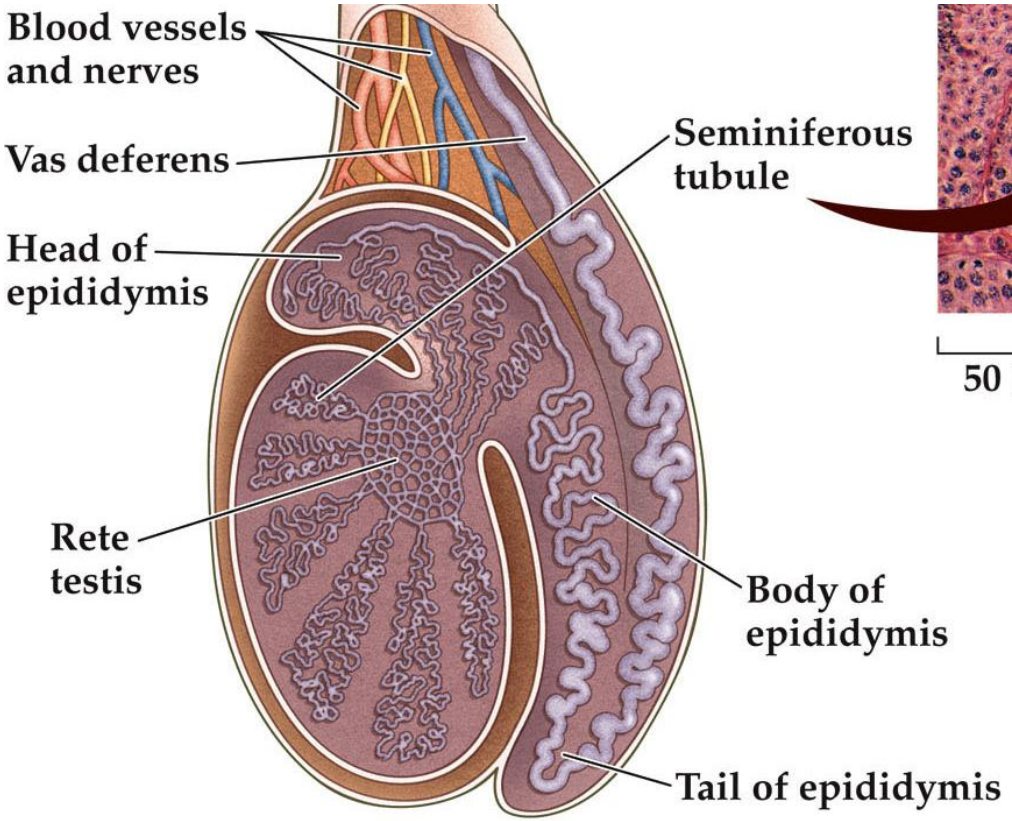
Permanent Methods for Males

Surgical or Chemical Vasectomy

Surgical method is not commonly used in domestic horses, but has been used successfully in field conditions with wild stallions

Chemical Vasectomy

Various chemicals have been used to cause scarring in the tubules of the epididymis, blocking passage of sperm



Outcomes of Vasectomy

If performed correctly, 100% effective after a lag-time of about 6 weeks (sperm already in tract must be cleared)

"Incorrect" surgery:

- Vet without vasectomy experience may cut the nerve bundle instead of the vas deferens
- Testis side of the cut must be closed or exteriorized to prevent re-canalization

"Incorrect" injection of chemical agent:

- Must inject into the lumen of the epididymal tubule:
  - to be successful at blocking sperm passage
  - to prevent schlerosis of scrotal tissue

Outcomes of Vasectomy

No effect on behavior

- Treated stallions should compete equally with intact stallions to acquire and hold harems
- Mares in harems headed by treated stallions should not conceive

Caveat: mares in bands with an intact subordinate stallion may eventually conceive

To reduce that possibility, all stallions gathered, up to the target number recommended by modelling, should be treated

Also, if pregnant mares from other harems with intact stallions transfer harems during gestation, there may be foals born in harems with treated male(s)

Balanced if treated stallion's mares transfer to non-treated harem

Reversible Methods for Males

Steroid Hormones

- High-dose testosterone appeared effective in about 75% of stallions treated
- No behavioral or physiological data were collected by which to assess side effects
- Generally, high-dose androgen is associated with heart and circulatory effects as well as increased aggression
- As for females, steroid hormone treatment is unlikely to be approved by EPA

GnRH Vaccines

To be effective at eliminating spermatogenesis, testosterone must be completely suppressed

As discussed above, the Committee felt that the elimination of testosterone and, thus, of male-typical behavior makes this method inappropriate

GnRH Agonists

As for GnRH vaccines, to be an effective contraceptive, testosterone must be completely suppressed, eliminating male-typical behavior

SOME ADDITIONAL FACTORS TO CONSIDER

Extending the breeding season

Methods that allow mares to continue cycling and stallions to maintain libido while preventing pregnancy will result in continued estrous cycles through the breeding season, April or May through August or September (depending on location)

Implications of extended breeding season

- The estrous cycle is about 3 weeks, with estrus lasting almost 1 week
- Thus, to maintain harems, stallions must spend most of those months guarding, courting and mating
- These activities expend energy and require diversion of time from grazing, potentially putting stallions at a disadvantage going into winter
- The single study of vasectomized stallions did not find a problem, but drought could exacerbate the effect

Identifying the most promising methods

Criteria

- Efficacy
- Duration of effect
- Availability
- Delivery method
- Side effects
  - Safe
  - Preserve behavioral system

Methods that met the most Criteria

Female-Directed

- PZP products
- GonaCon

Male-Directed

- Chemical vasectomy

Method	Advantages	Disadvantages
PZP-22 and SpayVac®	Research and application in both captive and free-ranging horses	Capture needed for hand injection of PZP-22
	Allows estrous cycles to continue so natural behaviors are maintained	Extended breeding season requires males to defend females longer
	High efficacy	With repeated use, return to fertility becomes less predictable
	Can be administered during pregnancy or lactation	Out-of-season births are possible

Method	Advantages	Disadvantages
Chemical Vasectomy	Simpler than surgical vasectomy	Requires handling and light anesthesia
	Permanent	Permanent
	No side effects expected	Only surgical vasectomy has been studied in horses, so side effects of the chemical agent are unknown
	Normal male behaviors maintained	Extended breeding season requires males to defend females longer and may result in late-season foals if remaining fertile males mate
	Should have high efficacy	Only surgical vasectomy has been studied in horses, so efficacy rate is unknown

Method	Advantages	Disadvantages
GonaCon™ for Females		Capture may be needed for hand injection of initial vaccine and any boosters
	Effective for multiple years	Lower efficacy than PZP-vaccine products, especially after first year
	Sexual behavior exhibited	Sexual behavior may not be cyclic, but ovulation appears to be blocked
	Social behaviors not affected in the single field study	Should not be administered during early pregnancy because abortion could occur
		Few data for horses

Behavior	PZP	GonaCon™ for females	Vasectomy
Male sexual	Increase or no change reported	No change reported	Longer breeding season
Female sexual	Increase or no change reported	Decrease or no change reported	Longer breeding season
Social structure	Possible decrease in band stability	No change reported	No change reported
Activity budget	Females may graze less	No change reported	No change reported
Aggression	Males may defend females longer	No change reported	Males defend females longer
Spatial relationships	Females may spend more time near male	No change reported	No change reported

Caveat

None of the methods is a magic bullet

- All require gathers
- PZP liquid can be used now, but has low efficacy with only one injection
- PZP-22 requires more development
- SpayVac and GonaCon should be tested further
- Chemical vasectomy needs validating in stallions